MANIPULATION OF PROBIOTICS FERMENTATION OF MILK BY *CINNAMON ZEYLANICUM, GLYCYRRHIZA GLABRA* OR *ALLIUM SATIVUM* AND THEIR EFFECTS ON INHIBITION OF *HELICOBACTER PYLORI* GROWTH *IN VITRO*

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ABSTRACT

Dairy products containing probiotics (e.g. *Lactobacillus* ssp. and *Bifidobacterium*) and certain herbs have inhibitory effects on the growth of *Helicobacter pylori*. The objectives of the present study were to determine the effects of herbs traditionally used for the treatment of gastric ulcer on yogurt fermentation characteristics, probiotic bacteria and the growth of *H. pylori in vitro*. *Cinnamon zeylanicum* (cinnamon), *Glycyrrhiza glabra* (licorice) or *Allium sativum* (garlic) was individually mixed with milk and the mixtures were fermented by probiotic bacteria to form herbal-yogurts. Changes in pH, titratable acididity, anti oxidant activity and the viable cell count of *Lactobacillus* ssp. and *Streptococcus thermophilus* were evaluated during refrigerated storage. The *in vitro* inhibition of *H. pylori* growth was determined using agar diffusion and minimum inhibitory concentration (MIC) methods. There were no significant differences in pH and TA between herbal-yogurts and plain-yogurt during fermentation and storage. Refrigeration up to 28 days increased ($p>0.05$) viable *Lactobacillus* ssp. counts to 15.8 x 10^6cfu/ml in the plain-yogurt but the presence of cinnamon, licorice or garlic tend to inhibit the increase ($p>0.05$) in herbal yogurts. Garlic-yogurt showed the least favored ($p>0.05$) for all characteristics tested for organoleptic properties. Water extract of cinnamon-yogurt and licorice-yogurt on day 7 of refrigerated storage showed the highest inhibitory effect against *H. pylori* strains UM-1, UM-2 and UM-3. An MIC of 3ml was effective for all *H. pylori* isolates by cinnamon and licorice yogurt water extracts, but only for *H. pylori* isolate UM-3 by garlic yogurt extract. Licorice-yogurt water extract had MIC at 1ml for isolate UM-1 and UM-2, whereas cinnamon-yogurt water extract had MIC at 2ml. Garlic-yogurt water extract had weak inhibition on *H. pylori*. The present *in vitro* findings indicate that yogurt and herbs under study can decrease the growth of *H. pylori*. These herbs could be used as food additives for the production of novel dairy products because of their unique functional attributes and potential mitigation on *H. pylori* growth.

Key words: Probiotics – Helicobater pylori – Medicinal Herbs – Herbal-yogurt – Lactobacillus ssp.

ABSTRAK

Produk-produk tenusu yang mengandungi probiotik (misalnya ssp. *Lactobacillus* dan *Bifidobacterium*) dan herba tertentu mempunyai kesan menghalang pada pertumbuhan *Helicobacter pylori*. Penelitian ini adalah untuk mengaji kesan dari herba yang digunakan secara tradisi untuk rawatan ulser perut ke atas ciri-ciri fermentasi yogurt, bakteria probiotik dan pertumbuhan *H. pylori in vitro*. *Cinnamon zeylanicum* (kayu manis), *Glycyrrhiza glabra* (licorice) atau *Allium sativum* (bawang putih) dicampur berasingan dengan susu dan campuran difermentasikan oleh bakteria probiotik untuk membentuk yogurt herba. Perubahan pH, jumlah asid tertitrasi, aktiviti anti oksidan dan jumlah sel hidup *Lactobacillus* ssp dan *Streptococcus thermophilus* dinilai selama simpanan berpendingin. Perencatan pertumbuhan *in vitro H. pylori* ditentukan dengan menggunakan kaedah difusi agar dan kepekatan minimun perencatan (MIC). Tidak ada perbezaan yang signifikan pada pH dan TTA antara yogurt herba dan yogurt kawalan selama fermentasi dan simpanan berpendingin. Pendinginan sehingga 28 hari meningkat ($p> 0.05$) *Lactobacillus* ssp ke jumlah 15.8 x 10^6cfu/ml untuk yogurt kawalan namun kewujudan kayu manis, licorice atau bawang putih cenderung menghalang peningkatan ($p> 0.05$) pada yogurt herba. Yogurt bawang putih adalah paling tidak disukai ($p> 0.05$) untuk semua ciri-ciri diuji untuk sifat organoleptik. Ekstrak air yogurt kayu manis dan yogurt licorice pada 7 hari simpanan berpendingin menunjukkan kesan penghambatan yang tinggi terhadap strain *H. pylori* UM-1, UM-2 dan UM-3. Extrak yogurt kayu manis dan yogurt licorice pada MIC 3ml adalah berkesan merancat semua isolat *H. pylori* tetapi hanya pada MIC 3ml bagi ekstrak yogurt bawang putih. Ekstrak air yogurt licorice pada kepekatan 1ml berupaya merencat UM-1 dan UM-2, sedangkan air ekstrak yogurt kayu manis mempunyai MIC 2ml. Ekstrak air yogurt bawang putih menghalang pertumbuhan *H. pylori*. Yogurt dan herba-herba yang diteliti secara *in vitro* dapat merencat pertumbuhan *H. pylori*. Tumbuh-tumbuhan ini boleh digunakan sebagai makanan tambahan untuk pengeluaran produk tenusu baru kerana sifat unik berfungsi dan potensinya mengekang pertumbuhan *H. pylori*.

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LIST OF ABBREVIATIONS

BF	Before Fermentation
µl	Microlitre
ml	Millilitre
µg	Microgram
mg	Milligram
nm	Nanometer
mm	Millimeter
rpm	Revolution Per Minute
LAB	Lactic Acid Bacteria
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
TA	Titratable Acidity
MIC	Minimum Inhibitory Concentration
DDM	Disk Diffusion Method
cfu	Colony Forming Unit
dH_2O	Distilled Water
BHIB	Brain Heart Infusion Broth
S. thermophilus	*Streptococcus thermophilus*
L. acidophilus	*Lactobacillus acidophilus*
H. pylori	*Helicobacter pylori*
GI	Gastrointestinal